

MINIREVIEW

Nocardiosis: Review of Clinical and Laboratory Experience

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Members of the genus *Nocardia* are associated with the group of microorganisms known as the aerobic actinomycetes and belong specifically to the family *Mycobacteriaceae*. The nocardiae contain tuberculostearic acids but differ from the mycobacteria by possession of shorter-chained (40- to 60-carbon) mycolic acids. They have a type IV cell wall, characterized by a peptidoglycan made up of *meso*-diaminopimelic acid, arabinose, and galactose (15). The systematics of this group of organisms originated from intuitive principles based on microscopic morphology and phenotypic characterization. The nocardiae are gram-positive, bacillary, branching bacteria whose hyphae often fragment to coccobacillary forms. Recent application of modern taxonomic procedures, inclusive of more extensive phenotypic evaluation, molecular characterization, and numerical taxonomic methods, has expanded our knowledge of their phylogenetic relatedness and taxonomic status (9, 13, 15). The taxonomy within the genus *Nocardia* is changing rapidly as the recognition and description of new species continue. As expected, there are differences of opinion as to the number of validly described species within the genus at this time, with recent publications citing from 22 to 30 such valid species (3, 14). Although a large number of species have been characterized both phenotypically and genotypically within the genus, the genotype remains greatly heterogeneous and will continue to evolve (3, 14). Sixteen species have been implicated in human infections (Table 1); but the geographic prevalence of each may change dramatically throughout the world, and some are uncommon. The species found most frequently in Arizona (Table 2) may vary substantially from those isolated in other parts of the United States.

NATURAL HABITAT AND EPIDEMIOLOGY

Nocardiae are found extensively worldwide and are saprophytic, making up an important component of the normal soil microflora and often being associated with water. They may also be associated with decomposing plant material, dust, and air (3, 15). As a species, *Nocardia asteroides* sensu stricto type VI is distributed evenly throughout the United States. *N. farcinica* is also found evenly throughout the United States, although it is less prevalent than *N. asteroides*. The distribution of other species varies regionally. *N. nova* is less commonly

isolated in the Southwest (authors' personal observations). Frequently, the term "*N. asteroides* complex" is used to include *N. asteroides* sensu stricto type VI, *N. farcinica*, *N. nova*, and more recently, *N. abscessus* because earlier reports failed to differentiate between the four species (3, 15). *N. brasiliensis* is associated with tropical environments, although in the United States it is the second most common isolate and has a higher prevalence in the southwestern and southeastern regions (3, 11, 15). *N. otitidiscaviarum* has infrequently been recovered from soil throughout the world. As with many other rarely reported nocardial species, the specific natural habitat of *N. transvalensis* has not yet been identified (3, 11, 15).

The majority of nocardial infections in the United States are acquired through inhalation (3, 15). It is the authors' personal observation that in the United States the overall number of nocardial infections seems to be greatest in association with the dry warm climates of the Southwest (Table 2). It may be that the dry, dusty, and often windy conditions in that region facilitate the aerosolization and dispersal of fragmented nocardial cells and enhance their acquisition via the respiratory route. A smaller number of infections are caused by traumatic introduction of organisms percutaneously. Normally, primary infections with *N. brasiliensis* and *N. otitidiscaviarum* in an immunocompetent host are associated with implantation via a foreign object. It is now known that many of the invasive infections thought to have been caused by *N. brasiliensis* were actually caused by a more recently recognized species, *N. pseudobrasiliensis* (17). Newly recognized species such as *N. africana*, *N. paucivorans*, and *N. veterana* have also been reported to cause disease in humans, although little is known of their epidemiology (6, 8, 13).

Nocardial infections are not thought to be transmitted from person to person and are not usually acquired nosocomially (15). However, there have been rare reports of interesting clusters of patients infected with identical strains of nocardia while occupying beds in close proximity on hospital wards. In such cases nosocomial acquisition was probable. It was difficult to ascertain, however, whether the infections were primary acquisitions from a common environmental source (environmental transmission) or secondary acquisitions from an initially infected patient (person-to-person transmission).

SPECTRUM OF DISEASE

Nocardiosis is usually an opportunistic infection and most commonly presents as pulmonary disease. The majority of patients with clinically recognized disease have underlying debil-

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TABLE 1. Species of the genus *Nocardia* associated with infections in humans^a

Association and species
Commonly associated ^b
<i>Nocardia asteroides</i> sensu stricto type VI
<i>Nocardia brasiliensis</i>
<i>Nocardia farcinica</i>
<i>Nocardia nova</i>
Less commonly or infrequently associated ^b
<i>Nocardia otitidiscaviarum</i>
<i>Nocardia pseudobrasiliensis</i>
Rarely associated or prevalence not established ^b
<i>Nocardia abscessus</i>
<i>Nocardia africana</i>
<i>Nocardia brevicatena</i> complex
<i>Nocardia carneae</i>
<i>Nocardia paucivorans</i>
<i>Nocardia transvalensis</i> (<i>N. asteroides</i> type IV)
<i>Nocardia transvalensis</i> (sensu stricto)
<i>Nocardia transvalensis</i> (new taxon I)
<i>Nocardia transvalensis</i> (new taxon II)
<i>Nocardia veterana</i>

^a Compiled from previous reports (3, 9, 14).^b Prevalence may vary by geographic location.

itating factors (3, 11, 15). Arguably, the most common condition predisposing the patient to nocardiosis is underlying chronic lung disease, often in association with long-term corticosteroid therapy (authors' unpublished observations). In a review of 16 patients with nocardiosis admitted to Banner Good Samaritan Medical Center, Phoenix, Ariz., over a 1-year period, nearly 75% had an underlying chronic pulmonary condition. Other predisposing conditions include diabetes mellitus, hematologic and other malignancies, transplantation, and AIDS. The authors estimate that less than 10% of patients with nocardiosis have no definable underlying predisposing factor. Healthy hosts with nocardial infections often have undergone percutaneous trauma and soft tissue inoculation (3, 4, 15).

The majority of primary cases present as pulmonary disease, although traumatically induced local abscesses occur as well. Dissemination from the lungs may be manifested as bacteremia, empyema, brain abscess, pericarditis, synovitis, and soft tissue infection (Table 3). Peritonitis and corneal ulcers have been described. Typically, nocardiosis is characterized by an acute inflammatory response terminating in necrosis and ab-

TABLE 2. Isolates of *Nocardia* spp. recovered from individual patients in the Phoenix cosmopolitan area between 1998 and 2002

Isolate	No. of isolates by yr					
	1998	1999	2000	2001	2002	Total
<i>N. asteroides</i> complex	42	67	68	65	65	307
<i>N. brasiliensis</i>	8	5	4	5	18	40
<i>N. farcinica</i>	2	8	6	13	5	34
<i>N. otitidiscaviarum</i>	1	1	1	1	2	6
<i>N. transvalensis</i>					1	1
<i>Nocardia</i> species	11	13	21	10	12	67
Total	64	94	100	94	103	455

TABLE 3. Individual source or specimen type of 470 *Nocardia* isolates recovered within the Phoenix, Ariz., area between 1998 and 2002

Isolate (no.)	No. of isolates by source or specimen type ^a						
	Resp	Blood	CNS	Body fluid	Wound	Ocular	Unkn
<i>N. asteroides</i> complex (319)	257	6	2	4	24	2	24
<i>N. brasiliensis</i> (44)	5			1	34	1	3
<i>N. farcinica</i> (34)	19	4	3		7	1	0
<i>N. otitidiscaviarum</i> (6)	5				1		
<i>Nocardia</i> species (67)	45	3	2	3	4		10
Total	331	13	7	8	70	4	37

^a Resp, respiratory tract, includes sputum and sinus ($n = 3$); CNS, central nervous system, includes cerebrospinal fluid specimens ($n = 5$) and brain biopsy specimens ($n = 2$); Body fluid, includes peritoneal ($n = 5$) and synovial ($n = 3$) fluids; Wound, includes soft tissue, lymph node and surgical wound; Unkn, unknown, includes unknown sources for isolates submitted from outside laboratories for susceptibility testing alone.

cess formation; granulomas are not normally formed (3, 4, 15).

In the United States most human infections are caused by *N. asteroides* sensu stricto type VI, *N. farcinica*, *N. nova*, *N. brasiliensis*, *N. otitidiscaviarum*, and possibly, *N. pseudobrasiliensis*. *N. transvalensis*, *N. africana*, *N. brevicatena*, and the newly described species are rarely encountered at this time. Members of the *N. asteroides* complex primarily cause pulmonary disease and, except for *N. nova*, are prone to extrapulmonary dissemination. Dissemination is especially prevalent with *N. farcinica* (3, 4, 15, 16) (Table 3). *N. brasiliensis* and *N. transvalensis* typically produce localized infection induced by an abrasion, although the latter species is uncommon in the United States. *N. brasiliensis* can be associated with a lymphocutaneous sporotrichoid presentation (Fig. 1).

DIAGNOSTIC METHODS

The clinical diagnosis of nocardiosis is difficult. Signs, symptoms, and radiologic studies may suggest the diagnosis but are not pathognomonic. Serologic diagnosis is unreliable, and serologic tests are not available commercially. The evaluation of appropriate specimens by smear and culture remains the prin-



FIG. 1. Sporotrichoid lymphocutaneous nocardiosis caused by traumatic implantation of *N. brasiliensis* in the middle finger.

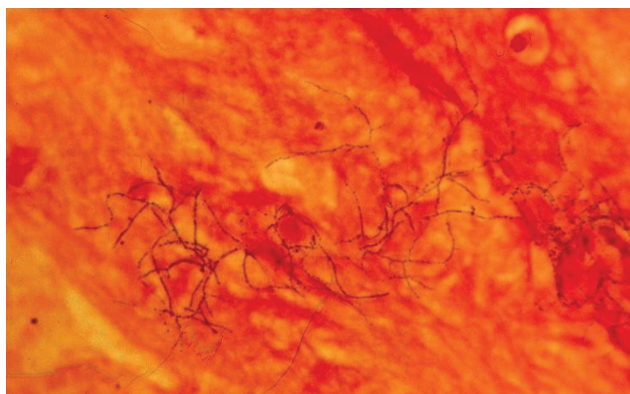


FIG. 2. Gram-positive beaded branching filaments of *N. asteroides* in a smear of sputum. Magnification, $\times 1,000$.

cial method of diagnosis. Detection in smears and isolation of nocardiae on primary and/or selective media are not usually difficult. This was demonstrated by an earlier study of 80 patients in whom nocardiosis was diagnosed by microbiologic or histopathologic techniques at Banner Good Samaritan Medical Center (M. A. Saubolle, unpublished observations, 1978 to 1989). Seventy-six of 80 (95%) patients studied had positive cultures, and in 50 of the 80 (64%) patients, infection was detected by Gram staining of infected material (Fig. 2). Of the 50 patients with specimens positive by Gram staining, only 26 (51%) were also positive by modified acid-fast staining (Fig. 3). Gram staining did not miss any acid-fast-positive specimens. Of the 76 patients from whom nocardiae were recovered, the isolates were recovered from 63 (83%) by culturing specimens on routine microbiologic media, and of these, 44 (70%) had Gram stain-positive primary specimens. Sixty-four of 76 (84%) patients had pulmonary involvement. Of the 51 of 64 patients from whom expectorated sputum was submitted for routine culture, nocardiae were detected in 40 (78%) by Gram staining and nocardiae were recovered from 45 (88%). Thus, Gram staining is the most sensitive method by which to visualize and recognize nocardiae in clinical specimens. The modified acid-fast stain is not reliable and should be used only to confirm the acid fastness of organisms detected by Gram staining.

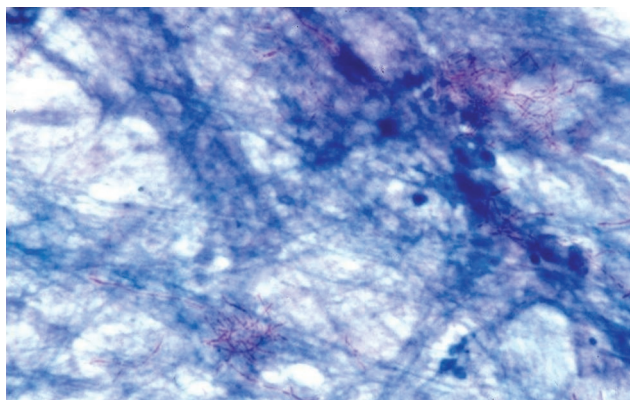


FIG. 3. *N. asteroides* filaments in a direct smear of sputum stained by the modified Kinyoun acid-fast method. Magnification, $\times 1,000$.

Nocardiae normally appear within 2 to 7 days on most routine bacteriologic media such as 5% sheep blood agar, chocolate agar, and BACTEC blood culture broth media. In cases in which suspicion of nocardia is high or in which the organism is visualized in specimens that are heavily contaminated, recovery may be optimized by the addition of selective media such as colistin-nalidixic acid agar, modified Thayer-Martin agar, and buffered charcoal-yeast extract (BCYE) and selective BCYE agars (3, 7, 15). The nocardiae seem to grow well on fungal media as well, including cycloheximide-containing agar, such as Mycosel. Some strains may, however, be inhibited by the gentamicin present in inhibitory mold agar. Media should be examined for up to 2 or 3 weeks for possible slowly growing nocardial strains, although the majority will be detected within the first several days of incubation. Recognition of the nocardiae can be optimized by seeing filamentous, white to yellow to orange colonies with aerial mycelia and delicate, dichotomously branched substrate mycelia with a dissecting microscope (15).

Although nocardiae are frequently isolated during culture for mycobacteria, procedures used for decontamination of contaminated specimens submitted for mycobacterial culture may be deleterious to the nocardia (12). The procedures used for specimens submitted for such culture should be supplemented with less selective procedures and culture media when nocardia is high on the differential diagnosis list.

CLINICAL RELEVANCE

The presence of nocardiae in the environment can lead to contamination and/or colonization of clinical specimens and can cause confusion over their clinical relevance. Nocardiae are rarely seen as contaminants in the laboratory, and each isolate must be carefully evaluated as to its clinical significance (4, 15). The presence of nocardia in normally sterile sites or on direct microscopic examination of potentially contaminated specimens, such as sputum, greatly increases the likelihood of the organism's role as an etiologic agent. Corticosteroid use significantly increases the clinical relevance of a sputum isolate.

IDENTIFICATION

Initial visualization of phenotypic colony coloration and morphology, together with the presence of aerial hyphae, with a dissecting microscope often provides initial clues to the genus of the isolate. Presumptive identification can be achieved if a filamentous, branched isolate stains with the carbolfuchsin modified acid-fast stain with a weak (0.5% to 1%) sulfuric acid decolorizing solution but not with the traditional Kinyoun acid-fast stain (15). Resistance to lysozyme differentiates *Nocardia* species from *Streptomyces* species. On occasion, examination of cell wall components by high-pressure liquid chromatography or thin-layer chromatography is needed for identification to the genus level. Identification to the species level may be more tedious and problematic. Originally, identification of the nocardial species was based on hydrolysis of casein, tyrosine, xanthine, and hypoxanthine. However, different stable susceptibility profiles among *N. asteroides* isolates showed that at least six unique species were identifiable (3, 9, 15, 17). Molecular as

TABLE 4. Biochemical reactions for timely, initial species separation within the genus *Nocardia*^a

Isolate	Reaction ^b								
	Galactose ^c	Glycerol ^c	Trehalose ^c	Adonitol ^c	Esculin	Opacification of 7H11 agar	Acetamide utilization	Growth at 45°C	AF ^d
<i>N. asteroides</i> sensu stricto	=	+	=	=	=	=	=	V	=
<i>N. transvalensis</i> (<i>N. asteroides</i> IV)	+	+	+	=	=	=	=	=	=
<i>N. farcinica</i>	=	+	=	=	+	+	+	+	=
<i>N. otitidiscaviarum</i>	=	+	+	=	+	=	=	V	=
<i>N. brasiliensis</i>	+	+	+	=	+	=	=	=	=
<i>N. pseudobrasiliensis</i>	+	+	+	=	+	=	+	=	=
<i>N. transvalensis</i>	+	+	+	+	=	=	=	V	=
<i>N. brevicatena</i>	=	=	+	=	+	=	=	V	=
<i>N. nova</i>	=	=	=	=	=	=	=	=	+

^a Supplemental testing is sometimes needed to resolve overlaps. Susceptibility testing, hydrolysis, or utilization of different carbon substrates may be used. The table was compiled in part with data from previous reports (3, 9, 15).

^b =, negative reactivity; +, positive reactivity; V, variable reactivity.

^c API 20C assimilation.

^d AF, arylsulfatase.

well as further phenotypic studies of the species confirmed their disparity and uniqueness. At present, molecular methods used to successfully identify the nocardiae to the species level include restriction endonuclease analysis of an amplified portion of the 16S rRNA gene, restriction fragment length polymorphism analysis of the amplified *hsp* gene, and sequencing methodologies, such as sequencing of the 16S rRNA or DNA (5, 10, 14, 18). Unfortunately, such molecular studies are often limited to research-oriented laboratories and are rarely performed in routine clinical laboratories. Although combinations of phenotypic and genotypic characterization are most successful in identifying all nocardial species, the majority of the frequently isolated, clinically relevant species can be initially identified by the use of a number of phenotypic characteristics. Use of arylsulfatase, acetamide utilization, gelatin liquefaction, growth patterns at 35 and 45°C, opacification of 7H11 agar, and reactions with a number of commercial systems, together with susceptibility profiles, can be used to identify most isolates within a reasonable time frame of less than 7 days. Table 4 provides a simple guide for the preliminary identification of the nocardiae in the clinical laboratory. Additional testing may be needed for isolates not keying out with the limited number of characteristics. The reader is referred to previous reports (3, 9, 15) for more extensive summaries and flowcharts.

THERAPY AND SUSCEPTIBILITY STUDIES

Sulfa-containing antimicrobials remain the drugs of choice and may improve survival when used alone or in combination with other antimicrobials (3, 4, 15). Primary agents that have been used successfully are minocycline, amikacin, imipenem, and linezolid. Combination therapy with a sulfa-containing agent and one of the primary agents has been recommended for serious, systemic disease (4). The use of amikacin in combination with imipenem has also been suggested for serious infections. Other potentially efficacious choices include the extended-spectrum cephalosporins, amoxicillin-clavulanate, newer macrolides, other aminoglycosides, and the fluoroquinolones (3, 4, 15). The duration of therapy is uncertain, but it should be protracted because of the occurrence of considerable numbers of relapses after shorter courses of therapy (4).

Nocardia species can vary in their antimicrobial susceptibility patterns. Therapeutic efficacy in individual patients may depend on species identity and on in vitro susceptibility studies (3, 4, 15). Susceptibility testing should especially be considered in refractory cases. A standard for susceptibility testing by broth microdilution and with cation-supplemented Mueller-Hinton broth has been approved by the NCCLS (19). Interpretative guidelines are provided in that publication. Disk agar diffusion, agar dilution, gradient strip agar dilution (E test), and BACTEC radiometric methods have all also been used for susceptibility testing (1, 2, 3, 15). Studies have shown rates of inter- and intralaboratory agreement and reproducibility above 90% between these methods (1, 2, 3, 15). Prospective clinical studies attempting to correlate the results of susceptibility testing to patient therapy and outcomes have not been systematically performed.

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REFERENCES

1. Ambaye, A., P. C. Kohner, P. C. Wollan, K. L. Roberts, G. D. Roberts, and F. R. Cockerill III. 1997. Comparison of agar dilution, broth microdilution, disk diffusion, E-test, and BACTEC radiometric methods for antimicrobial susceptibility testing of *Nocardia asteroides* complex. *J. Clin. Microbiol.* **35**: 847–852.
2. Biehle, J. R., J. R. Cavallieri, M. A. Saubolle, and L. J. Getsinger. 1994. Comparative evaluation of the Etest for susceptibility testing of *Nocardia* species. *Diagn. Microbiol. Infect. Dis.* **19**:101–110.
3. Brown, J. M., and M. M. McNeil. 2003. *Nocardia*, *Rhodococcus*, *Gordonia*, *Actinomadura*, *Streptomyces*, and other aerobic actinomycetes, p. 502–531. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 8th ed. American Society for Microbiology, Washington, D.C.
4. Burgert, S. J. 1999. Nocardiosis: a clinical review. *Infect. Dis. Clin. Pract.* **8**:27–32.
5. Conville, P. S., S. H. Fischer, C. P. Cartwright, and F. G. Witebsky. 2000. Identification of *Nocardia* species by restriction endonuclease analysis of an amplified portion of the 16S rRNA gene. *J. Clin. Microbiol.* **38**:158–164.
6. Eisenblatter, M., U. Disko, G. Stoltenberg-Didinger, H. Scherubl, K. P. Schaal, A. Roth, R. Ignatius, M. Zeitz, H. Hahn, and J. Wagner. 2002. Isolation of *Nocardia paucivorans* from cerebrospinal fluid of a patient with relapse of cerebral nocardiosis. *J. Clin. Microbiol.* **40**:3532–3534.
7. Garratt, M. A., H. T. Holmes, and F. S. Nolte. 1992. Selective buffered charcoal-yeast extract medium for isolation of nocardiae from mixed cultures. *J. Clin. Microbiol.* **30**:1891–1892.
8. Hamid, M. E., E. L. Maldonado, G. S. Sharaf Eldin, M. F. Mohamed, N. S.

- Saeed, and M. Goodfellow. 2001. *Nocardia africana* sp. nov., a new pathogen isolated from patients with pulmonary infections. J. Clin. Microbiol. **39**:625–630.
9. Kiska, D. L., K. Hicks, and D. J. Pettit. 2002. Identification of medically relevant *Nocardia* species with an abbreviated battery of tests. J. Clin. Microbiol. **40**:1346–1351.
10. Laurent, F. J., F. Provost, and P. Boiron. 1999. Rapid identification of clinically relevant *Nocardia* species to genus level by 16S rRNA gene PCR. J. Clin. Microbiol. **37**:99–102.
11. McNeil, M. M., and J. M. Brown. 1994. The medically important aerobic actinomycetes: epidemiology and microbiology. Clin. Microbiol. Rev. **7**:357–417.
12. Murray, P. R., R. L. Heeren, and A. C. Niles. 1987. Effect of decontamination procedures on recovery of *Nocardia* spp. J. Clin. Microbiol. **25**:2010–2011.
13. Pottumarthy, S., A. P. Limaye, J. L. Prentice, Y. B. Houze, S. R. Swanzy, and B. T. Cookson. 2003. *Nocardia veterana*, a new emerging pathogen. J. Clin. Microbiol. **41**:1705–1709.
14. Roth, A., S. Andrees, R. M. Kroppenstedt, D. Harmsen, and H. Mauch. 2003. Phylogeny of the genus *Nocardia* based on reassessed 16S rRNA gene sequences reveals underspeciation and division of strains classified as *Nocardia asteroides* into three established species and two unnamed taxons. J. Clin. Microbiol. **41**:851–856.
15. Saubolle, M. A. 2002. Aerobic actinomycetes, p. 1201–1220. In K. D. McClatchey (ed.), Clinical laboratory medicine, 2nd ed. Lippincott Williams & Wilkins, Philadelphia, Pa.
16. Torres, O. H., P. Domingo, R. Pericas, P. Boiron, J. A. Montiel, and G. Vazquez. 2000. Infection caused by *Nocardia farcinica*: case report and review of the literature. Eur. J. Clin. Microbiol. Infect. Dis. **19**:205–212.
17. Wallace, Jr., R. J., B. A. Brown, Z. Blacklock, R. Ulrich, K. Jost, J. M. Brown, M. M. McNeil, G. Onyi, V. A. Steingrube, and J. Gibson. 1995. New *Nocardia* taxon among isolates of *Nocardia brasiliensis* associated with invasive disease. J. Clin. Microbiol. **33**:1528–1533.
18. Wilson, R. W., V. A. Steingrube, B. A. Brown, and R. J. Wallace, Jr. 1998. Clinical application of PCR-restriction enzyme pattern analysis for rapid identification of aerobic actinomycetes isolates. J. Clin. Microbiol. **36**:148–152.
19. Woods, G. L., B. A. Brown-Elliott, E. P. Desmond, G. S. Hall, L. Heifets, G. E. Pfyffer, M. R. Plaunt, J. C. Ridderhof, R. J. Wallace, Jr., N. G. Warren, and G. F. Witebsky. 2003. Susceptibility testing of Mycobacteria, Nocardia, and other Actinomycetes. Approved standard M24-A, vol. 23, no. 18. NCCLS, Wayne, Pa.